0022-202X/80/7406-0389$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 74:389–391, 1980

Vol. 74, No. 6
Printed in U.S.A.

Binding of Bullous Pemphigoid Antibodies to Basal Cells

TAKEJI NISHIKAWA, M.D., SEIICHI KURIHARA, M.D., TAKASHI HARADA, M.D., AND HITOSHI HATANO, M.D.

Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Eight out of 12 serum samples from patients with bullous pemphigoid having basement membrane zone antibodies gave positive binding not only to the basement membrane zone but also to the basal cell membrane and/or cytoplasm as observed by complement immunofluorescence. Reaction of fluorescein labeled pemphigus vulgaris γ-globulins, binding mainly to the lower intercellular spaces of the epidermis, was greatly reduced by the prior incubation of high-titered bullous pemphigoid sera having the reactivity to the basal cells, while that of fluorescein labeled pemphigus foliaceus γ-globulins binding mainly to the upper and middle intercellular spaces, was not influenced by the prior application of these bullous pemphigoid sera. These results indicate that bullous pemphigoid antibodies are heterogenous and can be classified into 2 types and that some cross reaction is present between pemphigus antibodies and bullous pemphigoid antibodies having the reactivity to the basal cells.

Bullous pemphigoid (BP) is a chronic bullous dermatosis characterized by the presence of autoantibodies against basement membrane zone (BMZ) of the skin [1]. Using complement immunofluorescence (IF), we confirmed the complement-fixing capacity of BP antibodies and found by chance that some BP sera react not only with the BMZ but also with the cell membrane and/or cytoplasm of the proliferating basalioid cells of seborrheic keratosis (unpublished data).

In this study, we report that this positive binding of PB antibodies to the basal cells can be seen even in the BCM and/or BCC of normal human epidermis. According to their staining pattern, BP antibodies can be classified into 2 types, one with BMZ and basal cell binding and the other with BMZ binding only. In addition, the serological cross reactivity between BP and pemphigus antibodies will be reported.

MATERIALS AND METHODS

Serum

Twelve serum samples from patients with BP were included. Each serum having BMZ antibodies in titers ranging from 1:160 to 1:10,240 contained complement-fixing BMZ antibodies in titers from 1:20 to 1:640. Each serum was tested at 1:20 dilution after inactivation at 56°C for 30 min. Immunoglobulin classes of BMZ antibodies were shown to be IgG in each case with indirect IF with FITC labeled anti-human IgG, IgA, IgM respectively. Furthermore, the identity of the antibodies was confirmed by absorption of the sera with anti-human IgG.

Manuscript received May 29, 1979; accepted for publication November 28, 1979.

This work was presented at the 3rd Joint Meeting of the European Society for Dermatological Research and the Society for Investigative Dermatology, Inc., Amsterdam, June 10–14, 1979.

This work was supported by grants for autoimmune diseases from the Ministry of Health and Welfare of Japan, from Lydia O'Leary Memorial Foundation and from Professor Kato Memorial Research Fund for Physiology and Medicine.

Reprint requests to: Takeji Nishikawa, M.D., Department of Dermatology, Keio University School of Medicine, Shinjuku, Tokyo 160, Japan.

Abbreviations:
BCC: basal cell cytoplasm
BCM: basal cell membrane
BMZ: basement membrane zone
BP: bullous pemphigoid
FITC: fluorescein isothiocyanate
ICS: intercellular space
IF: immunofluorescence
PBS: phosphate buffered saline

Substrates

Normal human skin, obtained by skin biopsy was immediately frozen in dry ice-acetone, embedded in a Matrix (Lipshow, Detroit, U.S.A.), cut into 4–6 μm sections in a cryostat and were used unfixed.

Labeled Antisera

Commercially obtained FITC labeled antisera (Hyland Division, Travenol Lab., U.S.A.) were used for indirect IF and complement IF. The molar fluorescein protein ratio, specific antibody concentrations of the conjugates and dilutions employed were as follows: for IgG: 3.0, 2.0 mg/ml, 1:40; for IgM: 3.3, 1.9 mg/ml, 1:20; for IgA: 3.0, 1.2 mg/ml, 1:20; and for C3: 2.5, 2.5 mg/ml, 1:32. Specificity was confirmed by immunoelectrophoresis.

Immunofluorescence

Indirect IF was performed in the standard manner to test the sera of patients with BP for the presence of BMZ antibodies. Complement IF was assessed by the method described by Jordon et al [2,3]. Briefly, 3 drops of the inactivated BP serum were applied to the human skin sections and the same amount of fresh normal human serum diluted 1:2.5 with phosphate buffered saline (PBS) with Mg^{2+} and Ca^{2+} as a source of complement were overlaid on the sections. The sections were then incubated in a moist chamber at 37°C for 30 min. Following a PBS rinse, the sections were treated with the FITC rabbit antiserum against human C3 (Hyland) at 37°C for 30 min. The slides were rinsed again with the same buffer and mounted with coverslips in 50% glycerol-PBS. The specimens were examined with a Tiyoda fluorescence microscope equipped with Osram HBO mercury vapor lamp and dark field condensor. The specificity of complement IF was confirmed as described previously [3].

Preparation of FITC Labeled Pemphigus Antibodies

Conjugation of γ-globulins from 2 cases of patients with pemphigus (TY & MH) with FITC was performed through the courtesy of Dr. S. Kano of the Central Chemical Laboratory of Keio University Hospital according to the method of Wood, Thompson and Goldstein [4]. To take into consideration the relative concentration of antibodies within sera and conjugates, a system of units was used. One unit corresponds to the highest dilution giving positive IF.

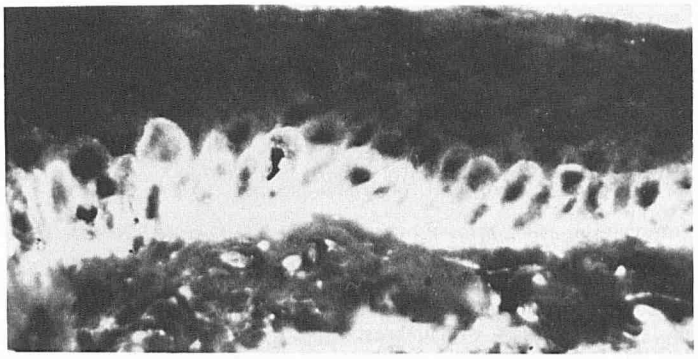
Blocking Immunofluorescence

The labeled pemphigus antibodies were used in blocking experiments to determine whether BP antibodies having ICS binding cross react with pemphigus antibodies. Skin sections were incubated with BP serum for 30 min at 37°C. After a PBS rinse for 15 min, they were stained with the FITC labeled pemphigus antibodies.

RESULTS

Binding of BP Sera with BMZ and BCM and/or BCC of Basal Cells

Of the 12 serum samples, 8 gave positive IF at both BMZ and BCM and/or BCC as observed by complement IF (Figure 1), though these sera showed no clear binding to the basal cells by the ordinary IgG indirect IF. Each BP antibody was of IgG type. This positive fluorescence was not demonstrated after the serum was incubated with excess amount of rabbit anti-human IgG. The positive reaction to the basal cells and BMZ was not



Complement immunofluorescence of a bullous pemphigoid serum (TH) giving basement membrane zone (BMZ) and basal cell staining. Positive fluorescence at BMZ, basal cell membrane and some basal cell cytoplasm (reduced from × 400).

TABLE I. *Correlation of intercellular binding by bullous pemphigoid antibodies with total IgG and complement-fixing IgG antibody titer*

No.	Patient	Basement membrane zone (BMZ) antibody titer		Site of positive staining[c]	
		Anti-IgG[a]	Anti-C3[b]	BMZ	Basal cell[d]
1	C.S.	1280	80	+	−
2	H.I.	1280	20	+	+
3	U.N.	640	80	+	+
4	K.T.	5120	80	+	+
5	S.A.	160	20	+	+
6	K.Ta.	640	40	+	−
7	H.N.	320	20	+	+
8	M.S.	320	20	+	−
9	T.H.	640	160	+	+
10	T.S.	160	20	+	+
11	N.I.	10240	640	+	−
12	A.Sh.	320	40	+	+

[a] Ordinary IgG indirect immunofluorescence (IF).
[b] Complement IF.
[c] Complement IF was performed at 1:20 serum dilution.
[d] Positive fluorescence at the basal cell membrane and/or basal cell cytoplasm.

TABLE II. *Characteristics of fluorescein isothiocyanate labeled pemphigus antibodies*

Source	Molar F/P	Titer	Positive staining	Dilution used
P. foliaceus (MH)	3.4	1:40	Upper & middle ICS[a]	2 units[b]
P. vulgaris (TY)	2.5	1:40	Lower ICS	2 units

[a] ICS: intercellular spaces.
[b] Unit: one unit is equal to highest dilutions giving positive staining.

demonstrated after the heat inactivation of the added fresh human serum. Blocking test using anti-human C3 also revealed the specificity of the C3 staining. This established that positive basal cell binding was caused by the reaction of C3 to the IgG class BMZ antibodies bound to the BCM and/or BCC. No clear correlation of basal cell binding by BP antibodies with the indirect IF antibody titer was observed as shown in Table I.

Binding of FITC Labeled Pemphigus Antibodies and the Serological Cross Reaction with BP Antibodies

The characteristics of FITC labeled pemphigus antibodies are shown in Table II. FITC labeled pemphigus foliaceus antibodies from case MH reacted mainly with upper and middle ICS as described by Bystryn, Abel, and DeFeo [5], while conjugated pemphigus vulgaris antibodies from case TY reacted with lower ICS only as described by Wood and Beutner [6]. Sera from patients with BP were assessed for their capability to block subsequent FITC labeled pemphigus antibodies. As shown in Table III, BP sera with basal cell binding (TH) blocked reaction of the subsequent application of FITC labeled pemphigus antibodies at higher concentration. This effect was more remarkable in the case of FITC labeled pemphigus vulgaris (TY) than pemphigus foliaceus (MH) antibodies. However, BP sera without basal cell binding (CS and MS) gave no effect on the subsequent staining of FITC labeled pemphigus antibodies from both forms (MH and TY) even at higher concentration.

DISCUSSION

Basement membrane zone of the skin is ultrastructurally divided into 4 components: (1) basal cell plasma membrane with its special attachment devices, hemidesmosomes, (2) the lamina lucida (or intermembranous space), (3) the basal lamina (basal membrane, basement membrane, lamina densa, adepidermal membrane) and (4) the sub-basal lamina fibrous elements including anchoring fibrils, dermal microfibril bundles and collagen fibers [7]. By immunoelectron microscopy BP antibodies are found to react with antigen located in the lamina lucida [8,9]. Furthermore, this antigen has been isolated from human skin [10] and the urine of a patient with BP [11]. Besides BP antigen, other components of the BMZ such as the basal lamina (collagen type IV) have been studied by immunological techniques [12,13].

Our present work clearly demonstrates that the majority of BP antibodies are reactive not only with BMZ but also with BCM and/or BCC using complement IF in which the stainability is usually amplified by the binding of C3 to each antigen-antibody complex. This observation verifies the possibility that antibodies can be present yet undemonstrable by the ordinary IgG indirect IF [14]. A similar phenomenon has recently been demonstrated in the case of autoimmune vitiligo in which antibodies against melanin producing cells could be detected with complement IF but not with the ordinary IgG indirect IF

TABLE III. *Blocking of the binding of fluorescein isothiocyanate (FITC) labeled bullous pemphigoid (BP) antibodies to the basal cell of the epidermis by pemphigus antibodies*

Case	BP-Ab titer	Positive staining	Unit used	FITC-M.H.[a]			FITC-T.Y.[b]		
				Upper	Middle	Lower	Upper	Middle	Lower
H.I.	1280	BMZ & BCM	7	+	+	−	−	−	−
			2	+	+	±	−	−	+
T.H.	640	BMZ & BCM	6	+	+	−	−	−	−
			2	+	+	±	−	−	+
C.S.	1280	BMZ	7	+	+	±	−	−	+
			2	+	+	±	−	−	+
M.S.	320	BMZ	5	+	+	±	−	−	+
			2	+	+	±	−	−	+
NHS[c]				+	+	±	−	−	+

[a] FITC-M.H.: fluorescein labeled γ-globulins from a patient with pemphigus foliaceus.
[b] FITC-T.Y.: fluorescein labeled γ-globulins from a patient with pemphigus vulgaris.
[c] NHS: normal human serum at 1:10 dilution for control.

[15]. Heterogeneity of the BP antibodies has not been pointed out to date, though some controversial observations have been reported about the immunoelectron microscopic findings of immunoglobulin deposition of BP skin [8,9,16]. Furthermore it was suggested from our study that BP antibodies were produced so as to reflect more of epidermal component of BMZ, since the positive correlation of reactivity of BP antibodies with pemphigus antibodies were demonstrated by the blocking experiment using FITC labeled pemphigus antibodies. Whether or not this basal cell fluorescence is related to the so-called basal cell cytoplasmic antibodies reported by van Joost and several others [17] remains to be solved, since basal cell antibodies are demonstrable without concomitant occurrence of antibodies to the BMZ.

Several immunochemical observation revealed close antigenic relationships between BMZ antigens and the epidermal antigens. Diaz et al [10] extracted PBS-soluble proteins from human skin by the treatment with 2N NaSCN which splits the epidermis at the level of the lamina lucida leaving the BP antigens attached to the epidermal sheet and isolated a protein moiety by gel filtration and ion exchange chromatography. This purified protein was able to block the staining of BMZ produced by BP antibodies. Interestingly, the same authors [18] found human saliva and urine contain substances which are reactive with both BP and pemphigus antibodies as detected by reverse passive hemagglutinations, suggesting pemphigus and BP antigens are immunologically related. Phylogenetic studies have also suggested that BP antigen, a more primitive antigenic moiety, gave rise to the pemphigus antigen during evolution [19]. Earlier IF studies by De Moragas, Winkelmann, and Jordon [20] revealed cytoplasmic fluorescence of basal cells in association with BMZ fluorescence using BP antibodies by the ordinary IgG indirect IF and concluded that their findings point to the elaboration of BMZ antigen by the basal or germinative cells of the epidermis. Bockendahl et al [21] suggested some relationships between the antigen of the BMZ and that of the ICS of epidermal cells from their careful histochemical analysis of the binding sites of BP antibodies. Our present study has provided stronger evidence that the antigenic property of BMZ as reflected by BP antibodies is more subject to the nature of epidermal cells on the BMZ. In other words, BMZ antigens may be of epithelial origin as described by Pierce et al [22] and therefore BP antibodies are able to bind with the antigens in the cytoplasm, BCM and ICS or basal cells on their way to the secretion to BMZ, although the intensity of fluorescence depends upon the amount of antigens or reactivity of BP serum. Precise localization of the substances should be further decided by the immuno-electron microscopy.

We are most grateful to those who kindly supplied the bullous pemphigoid sera used in the present study and Mrs. Yoshiko Fujii for her technical assistance.

REFERENCES

1. Jordon RE, Beutner EH, Witebsky E, Blumenthal G, Hale WL, Lever WF: Basement membrane zone antibodies in bullous pemphigoid. JAMA 200:751–756, 1967
2. Jordon RE, Nordby JM, Milstein H: The complement system in bullous pemphigoid III. Fixation of Clq and C4 by pemphigoid antibody. J Lab Clin Med 86:733–740, 1975
3. Nishikawa T, Harada T, Kurihara S, Sugawara M, Hatano H: Capability of complement fixation of pemphigus antibodies in vitro. Arch Dermatol Res 260:1–6, 1977
4. Wood BT, Thompson SH, Goldstein G: Fluorescent antibody staining. III. Preparation of fluorescein-isothiocyanate-labeled antibodies. J Immunol 95:225–229, 1965
5. Bystryn JC, Abel E, DeFeo C: Pemphigus foliaceus subcorneal intercellular antibodies of unique specificity. Arch Dermatol 110: 857–861, 1974
6. Wood GW, Beutner EH: Blocking-immunofluorescence studies on the specificity of pemphigus autoantibodies. Clin Immunol Immunopath 7:168–175, 1977
7. Briggaman RA, Wheeler Jr CE: The epidermal-dermal junction. J Invest Dermatol 65:71–84, 1975
8. Schaumburg-Lever G, Rale A, Schmidt-Ullrich B, Lever WF: Ultrastructural localization of *in-vivo* bound immunoglobulins in bullous pemphigoid—A preliminary report. J Invest Dermatol 64:47–49, 1975
9. Holubar K, Wolff K, Konrad K, Beutner EH: Ultrastructural localization of immunoglobulins in bullous pemphigoid skin, Employment of a new peroxidase-anti-peroxidase multistep method. J Invest Dermatol 64:220–227, 1975
10. Diaz LA, Calvanico NJ, Tomasi Jr TB, Jordon RE: Bullous pemphigoid antigen: Isolation from normal human skin. J Immunol 118:445–460, 1977
11. Diaz LA, Patel H, Calvanico, NJ: Bullous pemphigoid antigen II. Isolation from the urine of a patient. J Immunol 122:605–608, 1979
12. Heaphy MR, Jordon RE: Immunochemical studies of the human cutaneous basement membrane-anchoring fibril complex. J Invest Dermatol 69:513–515, 1977
13. Yaoita H, Foidart JM, Katz SI: Localization of the collagenous component in skin basement membrane. J Invest Dermatol 70: 191–193, 1978
14. Landry M, Sams Jr WM, Jordon RE: Bullous pemphigoid: Elution of in vivo fixed antibody. J Invest Dermatol 61:348–354, 1973
15. Hertz KC, Gazze LA, Kirkpatrick CH, Katz SI: Autoimmune vitiligo, Detection of antibodies to melanin producing cells. New Engl J Med 297:634–637, 1977
16. Masutani M, Ogawa H, Taneda A, Shoji M, Miyazaki H: Ultrastructural localization of immunoglobulins in the dermo-epidermal junction of patient with bullous pemphigoid. J Dermatol (Tokyo) 3:109–112, 1978
17. Van Joost T: Serum antibodies with affinity to the cytoplasm of epidermal basal cells, Immunopathology of the Skin, 2nd ed. Beutner, Chorzelski, Bean. John Wiley & Sons, New York, 1979, pp 473–488
18. Diaz LA, Patel H, Calvanico NJ: Immunologic relationship of pemphigoid and pemphigus antigens (abstr) Clin Res 26:489A, 1978
19. Diaz LA, Weiss HJ, Calvanico NJ: Phylogenetic studies with pemphigus and pemphigoid antibodies. Acta Dermatovener (Stockh) 58:537–540, 1978
20. DeMoragas JM, Winkelmann RK, Jordon RE: Immunofluorescence of epithelial skin tumors: II. *Basement membrane.* Cancer 25:1404–1407, 1970
21. Bockendahl H, Remy W, Remy B, Petersen G, Stüttgen G: Investigation on the binding sites of the basement membrane zone for pemphigoid antibodies in vitro: II. Immunohistochemical reaction of the reactive groups. Acta Dermatovener (Stockh) 57:15–21, 1977
22. Pierce Jr GB, Beals TF, Ram JS, Midgley Jr AR: Basement membranes: IV. Epithelial origin and immunologic cross reactions. Am J Pathol 45:929–961, 1964